

ORIGINAL ARTICLE

## Monitoring diseases of honey bees (*Apis mellifera*) in Russia

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Russia has favorable natural and climatic conditions and forage base for the beekeeping development. According to Russian Federal State Statistics Service, in 2016 the total number of bee colonies was 3.3 million, of which 3.1 million or 94% of the existing bee colonies were in private households with 78 regions out of 85 subjects of the Russian Federation being engaged in beekeeping. However, climate change, various biotic and anthropogenic factors have a negative impact on the state of the industry, manifested, first of all, in reducing the productivity of bee colonies, their quality, ability to resist diseases. Analysis of the results of research on bee colonies from 18 regions of the country (Arkhangelsk, Belgorod, Voronezh, Kirov, Leningrad, Moscow, Orenburg, Penza, Tomsk, Tula, and Tyumen regions; Altai, Krasnodar, Perm and Stavropol krais; republics of Mari El, Tatarstan, and Udmurtia) shows that the surveyed apiaries are affected by acariases, bacterioses, viroses, mycoses and microsporidiosis of bees. The study of bees and brood samples showed viruses such as deformed wing virus (DWV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), sacbrood virus (SBV), black queen cell virus (BQCV), Kashmir virus (KBV), Israeli acute paralysis virus (IAPV); bacteria: *Paenibacillus larvae larvae*, *Melissococcus pluton*, *Escherichia coli*; *Ascosphaera apis* fungus; mites *Varroa destructor*, *Acarapis externus*, *Acarapis woodi* Rennie; microsporidia *Nosema apis*, *Nosema ceranae*. Of the most common mixed infection-invasions of bees, the following can be identified: varroosis-nosemosis, varroosis-ascosphaerosis, varroosis-nosemosis-ascosphaerosis, varroosis-virosis (deformed wing virus), varroosis-virosis (sacbrood), varroosis-American foulbrood and European foulbrood. Studies conducted in Russia recent years are the basis for the development of a monitoring system for bee diseases in the country. To obtain objective data, joint efforts of scientists, the state veterinary service and owners of apiaries are needed. First of all, such kind of events should be held in regions with a large number of bee colonies (Altai, Krasnodar and Primorye krais; republics of Bashkortostan, Dagestan, Tatarstan, Udmurtia; Belgorod region.) Special attention should be paid to breeding farms.

**Keywords:** Honey bees *Apis mellifera*; diseases; monitoring; Russia

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### Introduction

Russia has favorable natural and climatic conditions and forage base for the beekeeping development. According to Russian Federal State Statistics Service, in 2016 the total number of bee colonies was 3.3 million, of which 3.1 million or 94% of the existing bee colonies were in private households with 78 regions out of 85 subjects of the Russian Federation being engaged in beekeeping (*Beekeeping of Russia in the Mirror of Russian and Foreign Statistics*, 2018). However, climate change, various biotic and anthropogenic factors have a negative impact on the state of the industry, manifested, first of all, in reducing the productivity of bee colonies, their quality, ability to resist diseases. Mass bee death (colony collapse disorder - CCD) in European and American countries led to the need for an international research association COLOSS (Prevention of honey bee Colony LOSSes), (Neumann & Carreck, 2010). COLOSS includes more than 900 members from 97 countries from research, veterinary, agricultural, and beekeeping organizations, centers involved in clarifying the reasons for the high mortality of bee colonies. One of the directions of such research is the study of new pathogens of honey bees. When analyzing the causes of death of bee colonies, along with the well-known causative agents of diseases of bees (*Varroa destructor*, *Nosema apis*, *Melissococcus plutonius*, *Penibacillus larvae larvae* etc.) new pathogens of the honey bee have been identified: the Israeli acute paralysis virus (IAPV), *Varroa destructor* virus, *Nosema ceranae*, and others. A significant role in this is played by the wide distribution of *Varroa destructor* and its negative impact on the bees resistance, which contributes to a sharp change in the epizootology and clinical manifestation of many diseases of these beneficial insects, the transfer of latent infections into evidently manifested diseases; its role in the formation of new bee pathogens is also not ruled out (Kulhanek K. et al., 2017; Van der Zee et al., 2014). The increased death of bee colonies in a number of regions of Russia, especially in the winter of 2016-2017, is reported by the mass media ("Portal Proufu.ru.", 2017; "Rosbalt News Agency", 2017). The country lacks the

overall health monitoring system of bees and the causes of their death, and therefore the research to identify the pathogens of honey bees conducted by Russian scientists in some regions are of high relevance.

## Materials and methods

The analysis was conducted, of the results of scientific studies of honey bees and veterinary reports from 18 regions of Russia for the period 2002-2018 for the presence of pathogens that cause the weakening and death of bee colonies throughout the world.

## Results and discussion

In Russia, an active study of bee viruses began in the 60s of the last century at the All-Union Research Institute of Experimental Veterinary Medicine. Scientists have developed serological methods for identification of pathogens, gave their descriptions, identified the deformed wing virus, sacbrood virus, acute bee paralysis virus, chronic bee paralysis virus, filamentovirosis (Batuev et al., 2008, 2010; Grobov et al., 2006). Studies of bees of the Carpathian breed of the Stavropol krai, Adygea, Moscow, and Tula regions with the use of RT-PCR for the presence of pathogenic viruses showed that in all of these regions the most common pathogens are the deformed wing virus (DWV), then the sacbrood virus (SBV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV). The Kashmir bee virus (KBV) and black queen cell virus (BQCV) were also identified. The presence of RNA-containing viruses (DWV, ABPV) in *Varroa destructor* was established, which confirms the possibility of bee viruses transfer by them (Kalashnikov & Udina, 2017). Studies conducted by (Maslennikova et al., 2017) established that in the Central, Northern and Southern federal districts the following viruses can be found: IAPV -4.2%, DWV -100%, BQCV -100%, KBV -37.5%. By frequency of occurrence of viruses, it was found that in the Northern Federal District four types of viruses are present on apiaries: the deformed wing virus, Israeli acute paralysis virus, black queen cell virus, Kashmir bee virus. In the Central Federal District, registered viruses are distributed as follows: black queen cell virus and deformed wing virus are present in all samples from apiaries of Voronezh, Belgorod, Penza and Ryazan regions; Israeli acute paralysis virus was identified only in the Belgorod region; Kashmir bee virus was found in the samples of Belgorod and Ryazan regions. In the Southern Federal District, three types of viruses have been detected: two of them (black queen cell virus, deformed wing virus) in the Rostov region and the Krasnodar krai; Kashmir bee virus -only in the Krasnodar krai. Thus, the conducted studies showed a very high burden of bee colonies by viruses of black queen cells - 100%, deformed wing virus -100% and a lower burden by Kashmir bee virus -37.5% and by Israeli acute paralysis virus -4.2%.

To study the spread of RNA-containing viruses, samples of biological material were taken from Udmurtian apiaries where at least 20% of the bee colonies died and at least 37% were affected by varroosis. It was established that the most common was the deformed wing virus (DWV -23.3%), which is most often found in bee colonies affected by *V. destructor*. With a lower frequency: the acute bee paralysis virus (ABPV -13.3%) and sacbrood virus (SBV -13.3%). The number of bee colonies in which a simultaneous presence of at least 2 viruses was detected was 13.3%, at least 3 viruses - 3.3%. Simultaneous infection with DWV and SBV viruses, as well as DWV and ABPV (Kalashnikov et al., 2013) was also present. To clarify the spread of other infectious diseases on the territory of the Republic, an analysis of veterinary reports for 8 years, as well as scientific research, were conducted, aimed at identifying varroosis, acarapidososis, noseosis, braulosis, other invasive, foulbrood, fungal diseases and bee pests. Particular attention was paid to the apiaries, which recorded the disappearance or death of bee colonies. As a result of the conducted studies, it was established that varroosis, noseosis, European foulbrood, ascospaerosis, which often take place in a mixed form, are widespread in the region. Out of 56 examined apiaries, 44.6% were affected by noseosis (20.1% of affected colonies), while in 93.3% of the samples the pathogen *Nosema apis* was registered, in 3.3% - *Nosema ceranae* and in 3.3% - the both microsporidia (Nepeivoda & Kolbina, 2014).

In Mari El in 2008-2009, an analysis of veterinary reports for the apiaries affected by varroosis and noseosis was conducted. It was found that in 15.6% of the 2333 samples examined *V. destructor* mites were detected, and in 9.0% - the causative agent of noseosis, *N. apis* (2008). In 2009, out of 2769 samples, the incidence of varroosis and noseosis in 8.3% and 5.9% of cases respectively was registered. Based on the data obtained, a system of measures for the prevention and therapy of studied diseases was developed (Makarov et al., 2010). The epizootic situation by noseosis in 7 districts of the Moscow Region was studied. During the period of 2009-2011, 1340 colonies were studied. From the 153 dead colonies, microsporidia *Nosema* were isolated in 22.9% of the cases, microsporidia *Nosema sp.* and mites *Varroa destructor* - in 36.6% of the cases. In a similar study of 4 colonies in the experimental apiary of All-Russian Research Institute for Veterinary Sanitation, Hygiene and Ecology using the RT-PCR method, noseosis causative agent *Nosema ceranae* was identified (Sokhlikov et al., 2012). Patterns of distribution and manifestation of especially dangerous infectious diseases of bees in the apiaries of four hothouse farms in the region were also studied. As a result of the conducted studies, the share of each disease among the general pathology in bee colonies was established. Varroosis was found in all bee colonies of hothouse farms. In the form of mono-infection, the disease was registered in 19% of colonies, and in the mixed form of infection -invasion in 81%. The most common mixed infection-invasion (ascospaerosis-varroosis) -in 34.5%, the second place in the occurrence of mixed infections-invasion (ascospaerosis-European foulbrood-varroosis) -in 23.4%; the third place was the mixed infection-invasion (European foulbrood-varroosis) -in 15.6%; the fourth was enterobacterial infection-invasion (salmonellosis-varroosis, hafniasis- varroosis) -in 7.6% of colonies. The data collected over 4 years indicate a stable presence in the colonies of infectious diseases of bees brood with an apparent, more than 2-fold, advantage of ascospaerosis over the European foulbrood (Maslennikova & Goleva, 2011).

In the Republic of Tatarstan, the epizootic state of apiaries for infectious and invasive diseases of bees was analyzed on the basis of veterinary reports of 2002-2009. According to the results of the analysis, 4 zones were established by the incidence of bee diseases: provisionally safe, mildly affected, moderately affected and severely affected. In the provisionally safe zone the incidence of ascospaerosis was 0-7%, in the mildly affected -8-14%, in the moderately affected -15-21 %, in the severely affected -from 22% and higher. By varroosis, provisionally safe zones include territories with a degree of incidence of up to 10%, mildly affected -from 11 to 20 %, moderately affected -incidence of 21-30%, severely affected -from 31% and higher. By noseimos, provisionally safe zone had incidence from 0 to 4%, mildly affected -from 5 to 8%, moderately affected -from 9 to 12% and severely affected - from 13 % and higher. Acarapidosis of bees was registered in 5 districts of the republic (Vasilevsky & Domolazov, 2011). According to the results of veterinary laboratories study in 2013-2015, 78.9 ± 3.5% of the of studied areas were affected by varroosis, 61.4 ± 4.4% by noseimos, 17.9 ± 2.1% by ascospaerosis (Shakirov & Nikitin, 2016). Studies of bee colonies on mycosis in the oil-producing regions of the Republic of Tatarstan in 2010-2012 showed a significant degree of incidence of ascospaerosis and aspergillosis. In the apiaries of Almetyevsky district in 2010, the level of bee colonies affected by ascospaerosis was 12.8%, and in 2012 it was 15%. In 2010, the incidence of bee aspergillosis was 10.8%, in 2011 - 11.2%, and in 2012 - 12.5%. The number of bee colonies with candidamycosis was 5.7% in 2010, 8.8% in 2011, and 11.9% in 2012. An analysis of the dynamics of the incidence of bees with melanosis indicates that in 2010 the number of colonies with queens affected by melanosis was 4.4%, in 2011 - 7.6%, and in 2012 - 10.7%. Such dynamics of the manifestation of mycoses persisted in the apiaries of Bugulma and Leninogorsk districts. The significant difference between the indicators of diseases of mycosis etiology in 2010 and 2012 researchers attribute to a sharp decline in the resistance of bee colonies (Nazarova, 2014). The studies were conducted, of the degree in which the bees of the Central Russian race (*Apis mellifera mellifera*) in the Perm krai and the introduced bees of the Carpathian race (*Apis mellifera carpathica*) are affected by noseimos. In the spring of 2013, samples of bees from 6 apiaries of the Perm krai (Perm, Uinsky, Kungursky districts) with a total of 384 bee colonies were studied. 56 bee colonies were studied, of which 41 were *Apis mellifera mellifera*, and 15 were *Apis mellifera carpathica* colonies. Bees of the Carpathian race showed a higher degree of damage, for example, 12 out of 15 bee colonies (80%) were infected, where the degree of infection varied from 70 to 100%. While on apiaries with Central Russian bees, the level of contamination of samples was 0-20% (Petukhov & Popov, 2014).

In the Orenburg region in the period of 2011-2013, bee colonies in apiaries of three different districts of the region were examined for the presence of causative agents of varroosis and noseimos of bees. An analysis was done, of the frequency of occurrence of infectious diseases of bees on the basis of data from the epizootic detachment of the region for diseases over a ten-year period from 2003 to 2013. The data of our own studies confirm a high degree of dissemination of varroosis and noseimos, while 75% of the bees sampled for research were found to have *N.apis*, and 100% had *V.destructor*. On the apiaries of the region episodic manifestations of salmonellosis were recorded. Within 10 years, the infection was registered 6 times at intervals 2003-2005, 2007-2008 and 2010, the incidence rate was 60% of the total sample. During the period under study, ascospaerosis appeared massively 3 times (2004, 2008, 2010), the incidence rate was 30%. Isolated cases of the causative agent of the American foulbrood - *P. larvae larvae* - and of colibacteriosis - *E.coli* - were also registered. The authors attribute the spread of diseases to the violation of veterinary and sanitary rules for keeping bee colonies, uncontrolled importation of bees from other territories (Ilina & Aladdina, 2014).

In 2009-2016, a study of the epizootic state of 206 apiaries in 12 districts of the Tyumen region was held. As a result of studying 1434 samples of dead bees, live bees and brood, apiaries affected by exo-acarapidosis (*Acarapis externus*), varroosis (*Varroa destructor*), noseimos (*Nosema apis*, *Nosema ceranae*), ascospaerosis (*Ascospaera apis*), European foulbrood (*Melissococcus plutonius*), sacbrood (SBV) were found. For the first time, on two apiaries, the previously unrecorded in the region causative agents of the American foulbrood and exo-acarapidosis were registered. Of the examined apiaries, 52.4% were noted as affected by varroosis, where the degree of affection of bee colonies was more than 1.0%, although varroa mites were found in almost all samples. A significant infection rate of bees by noseimos was recorded in 35.4% of apiaries. Bee colonies containing pathogens of exo-acarapidosis, ascospaerosis and European foulbrood were found in 11.0% of apiaries. The causative agent of the American foulbrood was found in two, and ascospaerosis - in 3 apiaries respectively. Analysis of the obtained data showed that the most common in the apiaries of the Tyumen region is a mixed infection-invasion (varroosis-noseimos). Joint research of scientific staff of the All-Russian Research Institute of Veterinary Entomology and Arachnology, the All-Russian Research Institute for Plant Protection and the Tyumen State University using the PCR method enabled in 2010 to identify, for the first time in Russia, two types of noseimos pathogens -*Nosema apis* and *Nosema ceranae* (Zinatullina et al., 2011). Studies conducted in 2012-2016 showed that out of 114 examined, 28.6-73.0% of apiaries with bee colonies were affected by noseimos. At the same time, 10.0-40.9% of the affected items revealed microsporidia *Nosema apis*, 40.9-72.7% - *Nosema ceranae*, and both pathogens were found in 18.8-30.0% of cases. Analysis of sources of infection of bees by *Nosema ceranae* indicates that the pathogen is registered in apiaries supplemented by imported bee colonies and queens, as well as colonies from other apiaries. (Zinatullina, 2016; Zinatullina et al., 2017). Samples of bees from 19 apiaries of the Kirov, Leningrad regions; Altai, Krasnodar, Stavropol, and Perm kraises were studied for noseimos. As a result of the research in all the above-mentioned regions, apiaries, affected by infection were found. At the same time, *Nosema apis* was registered in 4 apiaries, *Nosema ceranae* on 4 apiaries, both pathogens were isolated from samples of 6 apiaries. The results of the conducted studies confirm the tendency of displacement of the causative agent *Nosema apis* from the population of honey bees by the causative agent *Nosema ceranae* (Chen et al., 2008; Fries, 2010; Zinatullina et al., 2011).

In the Tomsk region, over the period of 2012-2016, 132 bee colonies from 68 apiaries of 11 districts were examined for noseimos. The disease was detected in 24 apiaries (35.3%) among those surveyed. *N.apis* spores were detected in 33 colonies (82.5%) of 18 apiaries (75.0%), *N.ceranae* was found in the bees of 3 colonies from 2 apiaries (8.3%), 4 colonies (10.0%)

in 4 apiaries (16.7%) revealed both pathogens. Thus, nosemosis was found in apiaries located in the territory of 5 districts out of 11 surveyed. At the same time, most of the infected bee colonies lived in the apiaries of the southern districts of the Tomsk region. Among the studied apiaries of the northern regions, only one apiary was registered with *N. apis*. The authors believe that the widespread spread of nosemosis in the apiaries of the south of the region may be due to more developed beekeeping and the active importation of bee colonies from the southern regions of Russia and from the neighboring countries (Kazakhstan, Uzbekistan, etc.) (Ostroverkhova et al., 2016). A retrospective analysis was carried out, of the contamination by nosemosis of stored samples of honey bees, collected from 74 colonies from 18 apiaries of 10 districts of the Tomsk region in the period of 2008-2012, as well as 10 samples from 2 isolated apiaries of the Krasnoyarsk krai, where for a long time the import of bee colonies and queens from other territories was not conducted. As a result of the studies, nosemosis pathogens were identified in 42 bee colonies, in that in 10 bee colonies (23.8%) only *N. apis* and in 10 other colonies (23.8%) *N. ceranae* were found, in the remaining (52.4%) both pathogens were present. In two isolated apiaries of the Krasnoyarsk krai, in the majority of the colonies studied, both pathogens were found with a predominance of *N. ceranae*. Based on the data obtained, the researchers suggest that *N. ceranae* is widespread in nature, which requires further study (Ostroverkhova et al., 2018).

The presented research results were obtained during the period of 2002-2017, mainly in the framework of scientific research conducted by Russian scientific and educational institutions in accordance with the research plans, and data of the veterinary service that is in the public domain. Based on the current situation with the diseases of honey bees in the world, the main focus of scientific research is aimed at identifying the causative agents of viral infections and microsporidia *Nosema ceranae* using modern methods of identifying pathogens.

Analysis of the results of research of bee colonies from 18 regions of the country (Arkhangelsk, Belgorod, Voronezh, Kirov, Leningrad, Moscow, Orenburg, Penza, Tomsk, Tula, Tyumen regions; Altai, Krasnodar, Perm and Stavropol krais; republics of Mari El, Tatarstan, and Udmurtia) shows that the surveyed apiaries are affected by acariases, bacterioses, viroses, mycoses and microsporidiosis of bees. In the study of bees and brood samples, the following viruses were identified: deformed wing virus (DWV), acute bee paralysis virus, (ABPV), chronic bee paralysis virus (CBPV), sacbrood virus (SBV), black queen cell virus (BQCV), Kashmir bee virus (KBV), Israeli acute paralysis virus (IAPV); bacteria: *Paenibacillus larvae larvae*, *Melissococcus plutonius*, *Escherichia coli*, fungus *Ascosphaera apis*; mites: *Varroa destructor*; *Acarapis externus*, *Acarapis woodi*; microsporidia *Nosema apis*, *Nosema ceranae*. Of the most common mixed bee infections-invasions, the following can be distinguished: varroosis-nosemosis, varroosis- ascosphaerosis, varroosis-nosemosis-ascosphaerosis, varroosis-virosis (deformed wing virus), varroosis-virosis (sacbrood), varroosis-American foulbrood and European foulbrood.

The results obtained coincide with the data available in other countries. The need for a systematic monitoring of the health of bees is indicated by studies conducted in a number of European countries, including Ukraine (Fedoriak et al., 2017; Galatyuk et al., 2014), Spain, France, Germany, Switzerland, Denmark, Finland, Greece, Hungary, Holland, the United Kingdom, Italy, Serbia, Poland, Slovenia, Bosnia and Herzegovina, Sweden and others. Application of molecular genetic methods of identification of pathogens of honey bee *A. mellifera* allowed to detect two types of *Nosema* causing disease of bee colonies, accompanied by a sharp weakening and increased death of bees (Goblirsch, 2017; Higes et al., 2010; Natsopoulou et al., 2016). In the study of *Nosema* isolates from Finland, stored from 1986 to 2006, it has been established that *N. ceranae* has been present in Europe since 1998 and is more common than *Nosema apis* (Paxton et al., 2007). In the process of studying 28 samples from 108 *A. mellifera* bee colonies in three natural and geographical zones of Bulgaria (Southern, Northern and Western Bulgaria), it was found that bees from the northern part of the country had the highest level of infection (77.2%) by *Nosema ceranae*, while those from the mountainous parts (Rhodope, Southern Bulgaria) - only 13.9% (Hristov et al., 2017). Studies conducted in Slovenia showed high mortality of bee colonies with mixed invasion- infections caused by the *V. destructor* mite and the deformed wing virus (DWV), the chronic bee paralysis virus (CBPV), and *N. ceranae* and (DWV), *N. apis* and filamentous virus (FV), Y virus (BVY), and black queen cell virus (BQCV), (Toplak et al., 2013). Inspectors of a British national group in 2007-2008 monitored the pathogens of bees on problem apiaries of England and Wales. From the 406 samples of adult bees, the causative agents of viral infections (DWV, BQCV), microsporidia *N. apis*, *N. ceranae*, mites *A. woodi* and *V. destructor* were identified. (Budge et al., 2015).

*Nosema ceranae* is a widespread infection of the European honey bee *Apis mellifera* in the United States, as evidenced by studies of honey bee samples from 12 states between 1995 and 2007. The identification of *N. ceranae* in bees collected ten years ago indicates that *N. ceranae* is transferred from its original host *Apis cerana* to *A. mellifera* (Chen et al., 2008).

Studies of bee colonies in Iraq made it possible to isolate, for the first time, the causative agent of the American foulbrood, *Paenibacillus larvae larvae*. The study of 80 brood samples from apiaries from different regions of the Dohuk Governorate showed that the pathogen was identified in 16.2% of the samples studied, which were selected on 5 apiaries out of 7 surveyed. The results indicate a wide spread of American foulbrood in Iraq (Ayoub et al., 2013). It has been established that in the mountainous regions of Azerbaijan on apiaries there are present: the deformed wing virus, the black queen cell virus, the Kashmir bee virus, the Israeli acute paralysis virus and the microsporidium *N. ceranae* (Khanbekova et al., 2013).

In Mongolia, for the first time in 2015, 151 bee colonies from 9 apiaries located in 3 districts were examined for the presence of bee pathogens. Despite the high sensitivity of the methods used and a sufficient number of samples of the bee colonies studied, mites of *V. destructor*, *Nosema ceranae* and four viruses (BQCV, DWV, ABPV, CBPV) were found with different prevalence. Phylogenetic analyses of detected viruses show their clustering and European origin, thereby confirming the role of commercial transfer of bee colonies in the spread of pathogens. (Tsevegmid et al., 2016).

In Japan, the deformed wing virus (DWV), the black queen cell virus (BQCV), the acute paralysis virus (IAPV) and the sacbrood virus (SBV) are also recorded; however, the frequency of *A. ceranae japonica* virus infection is lower than in *A. mellifera* colonies



imported into the country. The first tracheal mite (*Acarapis woodi*) was found in dead bees *A. c. japonica*. These results demonstrate the infection of native honey bees with pathogens of other species of honey bees that circulate (are sold) around the world (Kojima et al., 2011).

Thus, the results of the studies presented in the article indicate a wide spread of the causative agents of bee diseases in the world and one of the reasons for this is the development of economic ties between countries.

## Conclusion

Studies conducted in Russia in recent years are the basis for the development of a monitoring system for bee diseases in the country. To obtain more objective data, joint efforts of scientists, the state veterinary service and owners of apiaries is needed. First, such kind of events should be held in regions with a large number of bee colonies (Altai, Krasnodar and Primorye kraies; republics of Bashkortostan, Dagestan, Tatarstan, Udmurtia; and Belgorod region). Particular attention should be paid to breeding farms.

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